Classification of Parkinson's Disease and Delineating Progression Markers from the Sebum Volatilome

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Abstract

Background: Parkinson's Disease (PD) has been associated with a distinct odour, which emanates from the skin and is strongest in sebum-rich areas. Thermal Desorption – Gas Chromatography – Mass Spectrometry (TD-GC-MS) has revealed volatile signatures from sebum that distinguish individuals with Parkinson's Disease (PD) from healthy controls. Here, we hypothesized that the sebum volatilome of individuals will alter as the disease progresses, since we have shown that endogenous metabolites do alter in abundance at different stages of the disease. We sought to determine features that could be used as progression markers.

Methods: Sebum was sampled from participants using cotton gauze and the headspace from these swabs was analysed directly with TD-GC-MS using established methods.[1,2] Supervised and unsupervised multivariate analysis was used to compare the relative abundances of significant volatile metabolites found for each phenotype. Our primary model was built on data from subjects with clinically established PD (n=46) and healthy controls (n=28) sampled at two sites. The volatilome profiles obtained for these PD and control cohorts were compared with the profile of participants with idiopathic REM sleep behaviour disorder (iRBD) to investigate metabolite changes in probable prodromal PD. A subset were also subjected to olfactory analysis. We also compared a subset of PD participants sampled at yearly intervals for a total of three years and built a subsequent model from this data. For all models the significant features were putatively annotated based on accurate mass and fragmentation patterns against spectral libraries.

Findings: Classification between PD and control validated our previously published work. Significant features were investigated, and the majority that were putatively annotated were alkanes and fatty acid methyl esters (FAMEs), with other metabolites identified as an aldehyde, purine, oleamide and tropinone. Data from iRBD samples were then included in the analysis and we found that samples from individuals with PD and with iRBD could be classified completely from each other and from control samples. We found 27 significant features whose abundance in samples from individuals with iRBD was intermediate between that found for PD and control samples. In olfactory analysis of a subset all PD samples were correctly identified, and 3/9 of the iRDB samples were classified PD. On clinical follow up 2 of these showed PD symptoms. Further, when analysing the volatilome from longitudinal PD sampling, almost a two-thirds of the significant features showed differential regulation over the three visits.

Interpretation: There are differential volatile metabolite signatures in sebum from PD and control participants even with low (n < 50) numbers of samples which is useful when comparing to rare and prodromal phenotypes. In this pilot investigation, we find that iRBD volatile metabolite signature can be separated from PD and control subjects using multivariate analysis, and almost two-thirds of the significant features can be seen to alter at disease classification stage, indicating possible early markers of disease and its progression. In longitudinal samples up or down regulation over 3 years also provides evidence for the presence of progression markers in sebum. When considering PD *vs*. control participants, more than 85% of the identifiable metabolites were putatively annotated as hydrocarbons

and fatty acid methyl esters (FAMEs). Our prior work indicates that these components arise from larger lipid molecules that decompose during ionisation in TD-GC-MS.[2] Validation of the change in the volatilome of individuals was provided by odour analysis.

Funding: We thank Michael J Fox Foundation (grant ref:12921) and Parkinson's UK (grant ref: K-1504) for funding this study and Community of Analytical and Measurement Sciences (CAMS) for supporting DT's research (CAMS2020/ALect/10). This work was supported by the BBSRC (award BB/L015048/1) for instrumentation used in this work and by a DTP to CW-D (project ref. 2113640). We also thank our recruitment centres for their enthusiasm and rigor during the recruitment process. We are very grateful to all the participants who took part in this study as well as PIs and nurses at the recruiting centres.

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder, with more than six million people affected worldwide and an expected two-fold increase in prevalence over the next generation.[3] The pathological hallmarks are neuronal α-synuclein positive inclusions and cell loss in the substantia nigra, other areas of the brain, and the peripheral autonomic nervous system causing the cardinal motor features of bradykinesia, rigidity and rest tremor as well as a plethora of non-motor symptoms.[4] Current diagnostic criteria for PD are anchored on clinical features, which overlap with other conditions and may result in initial misclassification.[5] Highly sensitive and specific tests, therefore, are a major need in PD - both to enhance diagnostic accuracy and to enable detection of the earliest disease stages where future disease-modifying therapies would be the most effective.[5]

Clinically manifested PD evolves from a prodromal stage characterised by the occurrence of a variety of non-motor symptoms, including anosmia, constipation, anxiety, depression, change in cognitive behaviour as well as REM-Sleep Behaviour Disorder (RBD).[6,7] Together with various risk markers, these have been used to define research criteria for prodromal PD. Within these criteria a polysomnographic diagnosis of idiopathic RBD (iRBD), characterised by loss of REM-atonia and jerks or dream-enacting behaviours during REM sleep confers the highest risk for future PD with a likelihood ratio of 130.[8]

PD has been associated with a distinct odour, strongest in sebum-rich areas. Sebum is a lipid-rich substance secreted by the sebaceous glands on the skin, which are most concentrated on the face and upper back. Increased sebum output (seborrhoea) and seborrheic dermatitis are well-known features of PD and changes in volatile profiles of sebum have recently been detected using Thermal Desorption – Gas Chromatography – Mass Spectrometry (TD-GC-MS).[1,2] Sebum is an alternative biofluid found on the skin and that can be obtained by non-invasive sampling, giving rise to advantages such as at-home sampling as well as low consumable and transport costs.

So far, TD-GC-MS has not been employed on cohorts showing prodromal PD symptoms, which would be important to understand if volatile profiles of sebum have potential as biomarkers for early diagnosis. In this study, we analysed sebum samples from PD and control cohorts finding good discrimination between their volatile metabolite profiles. We investigated features that contributed highly to the separation of phenotypes and putatively identified them using fragment spectra. We further analysed

samples from iRBD participants. We describe how odour and mass spectrometry markers permit classification between these three different phenotypes, and features where iRBD concentration lies between disease and control, indicative of possible biomarkers of early stages of disease. Further, we examined markers in samples collected longitudinally from PD participants at three annual timepoints to investigate possible progressive features.

Subjects and Methods

Sample Collection and Clinical Cohort

Subjects were recruited at two sites: the Movement Disorder and Sleep Disorder out-patient clinic, at the Department of Neurology, Medical University Innsbruck, Austria (Austria cohort) and the Department of Neurology at Salford Royal Foundation Trust (UK cohort). Clinic staff, patients' caregivers, and patients without neurodegenerative or ongoing dermatological disorders were invited to participate as healthy controls. Sebum samples were collected from the upper back of participants using sterile cotton-based gauze swabs (7.5 x 7.5 cm) by single individuals to avoid variance due to sampling. The sampling protocol is described in earlier work.[1,2]

The UK cohort consisted of people with PD (PwPD) (*n*=30) and control (*n*=19) participants. From 30 PD participants, 11 were sampled longitudinally three times in total, ~12 and 24 months after the first sampling. Controls were not included in longitudinal sampling. The Austrian cohort consisted of participants diagnosed as PwPD (*n*=16) and PSG-proven iRBD (*n*=9) as well as controls (*n*=9). Participant demographics are detailed in Table 1. Two iRBD subjects developed subtle parkinsonian signs after follow-up, but had been free of any such sign at the time of sampling. The majority of our PD and iRBD participants are male in line with the global prevalence rates.[9,10]

Table 1: *Participant demographic information and sample groups for analyses.*

Location/phenotype	***Participants***	***Sex (M:F)***	***Age mean (SD)***	***BMI mean (SD)***
UK/ PD Timepoint 1	30	5:1	63.83 (7.87)	27.01 (3.67)
*UK/ PD Timepoint 2 & 3**	11	11:0	60.09 (8.51)	27.42 (2.78)
UK/ Controls	19	10:9	63.89 (11.19)	26.37 (3.87)
Austria/ PD	16	3:1	64.63 (6.38)	25.77 (3.78)
Austria/ iRBD	9	8:1	69.89 (11.83)	27.93 (4.69)
Austria/ Controls	9	8:1	60.44 (4.80)	27.06 (3.61)

**consisted of same 11 PD participants sampled at ~12 and 24 months after first sampling*

Following collection, the gauze swabs were placed in Ziplok plastic bags, transported to the test site and stored at -80 ˚C until analysis. Dynamic headspace was analysed from all these samples in a randomised order. Raw data were deconvolved, annotated and converted to a data matrix consisting of 613 features. We interrogated these data. In summary, we investigated two key questions:

1. Do the components contributing to the VOC signature change as Parkinson's progresses?
2. Is there an VOC signature associated with iRDB and can it be distinguished from that of PD and of control participants with TD-GC MS?

Materials

Sterile gauze swabs (Arco, UK) and sample bags (GE Healthcare Whatman, UK) were used for sample collection. The headspace was collected into 20mL headspace vials (GERSTAL, Germany) and absorbed on to TENAX TA sorbent tubes and packed liners (GERSTAL, Germany). The solvents used for making system suitability mix were Optima LC-MS grade methanol (Fisher Scientific) and CHROMANORM absolute 99.8% ethanol (VWR Chemicals). The system suitability mix used to check for instrumental drift was a mix of seven compounds (Sigma-Aldrich): l (-)-carvone (27.0 μM), δ-decalactone (96.4 μM), ethyl butyrate (150.5 μM), ethyl hexanoate (30.5 μM), hexadecane (43.7 μM), nonane (83.07 μM), and vanillin (100.8 μM), all in MeOH: EtOH (9:1).

Analytical Setup

Samples were thawed and removed from bags before transference to headspace vials (20mL). Analysis was performed on an Agilent GC (7890A) coupled with MS (Agilent MSD 5975) equipped with a GERSTAL MPS handling system. Volatile analytes were preconcentrated by incubating samples at 80˚C for 10 mins. Analytes were trapped using 1L dry nitrogen purged through the headspace at 70 mL/min and onto the TENAX TA sorbent tube held above at 40˚C. The sorbent tube subsequently was transferred to the Thermal Desorption Unit (TDU) located in the GC inlet where it was held at 30˚C for 1 min before the temperature increased at 12˚C/s until 280˚C and held for 5 min. The Cooled Injection System (CIS) was held at 10˚C for 0.5 min before a 12˚C/s ramp up to 280˚C and held for a further 5 min. Analytes were separated on an Agilent VF-5MS column (30m x 0.25mm x 0.25um) with a flow of 1mL/min and a split ratio of 1:20. The temperature of the oven was held at 40˚C for 1 min then ramped at 25˚C/min to 180˚C, 8˚C/min to 240˚C and held for 1 min then 20˚C/min to 300˚C where it was held for 2.9 min. Positive pressure was added to the splitter plate at 1.6mL/min. The MSD operated in scan mode between *m/z* 30-800 with the source temperature held at 230˚C and quadrupole temperature held at 150˚C.

Olfactory Analysis

Samples (gauze swabs in plastic bags) of known phenotype were presented to the tester (an individual with hyperosmia- a heightened sense of smell) to train and familiarise. Once training was completed, blinded samples were presented to the tester in the presence of observers. The tester was asked to describe the strength and type of smell, which was recorded on a ranking scale with comments. The tester 'reset' their nose by smelling a scent refresher of their choice between samples and periodic breaks were taken if the tester was overwhelmed. A subset of samples were revisited twice at the testers request.

Data Processing and Statistical Analyses

Raw mass spectrometry data files were converted to .mzXML open format using ProteoWizard MSconvert.[11] Subsequently, .mzXML files were deconvolved in R using the eRah package[12] resulting in a data matrix with 613 deconvolved and putatively identified features. The features were assigned putative identifications using the GOLM and NIST 14.0 databases within eRah. Assignments of TMS derivatives, siloxanes or features with a match factor <70 were not considered and removed from further analyses. The final data output was a matrix of features and the relative feature intensity of these in each sample. In the absence of biological pooled QC, a mixture of standards was injected five times at the start of each batch, after every 5 sample injections and three times at the end of each batch. The instrument's stability was assessed using the relative standard deviation (%RSD) of the peaks from this system suitability mix. All peaks of the SST showed %RSD <20% and data quality were considered satisfactory. All data was normalised to the sum of the total ion count, log transformed and auto scaled prior to statistical analyses. We employed multivariate statistical analyses methods to analyse the mass spectrometry data as described in supplementary information S1. Sparse partial least squares discriminant analysis (sPLS-DA) was used to model data classifying between the studied cohorts described in the table below.

Table 2: *Description of three models generated to compare various phenotypes and samples included in each of those. For longitudinal analysis the age stated is based on that at the first visit.*

Comparison	***PD***	***iRBD***	***Control***
Parkinson's vs Control (Fig 1)	Age 64.11 (7.32) BMI 26.58 (3.72) Gender (M:F) 37:9	N/A	Age 62.79 (9.64) BMI 26.58 (3.72) Gender (M:F) 18:10
Parkinson's vs Control vs iRBD (Fig 2)	Age 64.63 (6.38) BMI 25.77 (3.78) Gender (M:F) 3:1	Age 69.89 (11.83) BMI 27.93 (4.69) Gender (M:F) 8:1	Age 60.44 (4.8) BMI 27.06 (3.61) Gender (M:F) 8:1
Parkinson's progression over 3 years (Fig 3)	Age 60.09 (8.51) BMI 27.42 (2.78) Gender (M:F) 11:0	N/A	N/A

Confounders

We investigated the confounding effects for participants' age, BMI, gender, alcohol consumption (units per week), smoking habits (cigarettes per week) and existing comorbidities. The common comorbidities within our cohorts were high cholesterol, hypertension, history of skin conditions, bone disease, thyroid conditions and heart conditions and we also investigated the likelihood of any confounding effects for all these (as described in supplementary info S2).

Results and Discussion

PD classification using sebum VOCs

We found 613 robust features from the TC-GC MS analysis of each of the participants. The sPLS-DA scores plot (Figure 1a) shows good clustering of the two phenotypes, which was cross validated and had an error rate of 0.2. From this analysis, of the 613 features, 85 were determined to be significant variables that enable classification between PD and control. Of these 85, 25 are seen to be upregulated with PD and 60 down regulated. Of these significant features, 28 could be assigned putative identifications at MSI level 2: seventeen as alkanes and seven as Fatty Acid Methyl Esters (FAMEs). Purine, tropinone, oleamide and hexadecanal were also identified and upregulated in the control samples. Lack of identification of the remaining significant features can be attributed to a lack of a suitable match in spectral libraries or possibly be due to chromatographic co-elution, or lack of significant precursor ions. Example expressions of annotated significant features are displayed in Figure 1b. This data validates our earlier observation that volatile compounds from sebum can distinguish PD from control, irrespective of geographical location of subjects. A list of all features found significant is shown in supplementary information table S1.

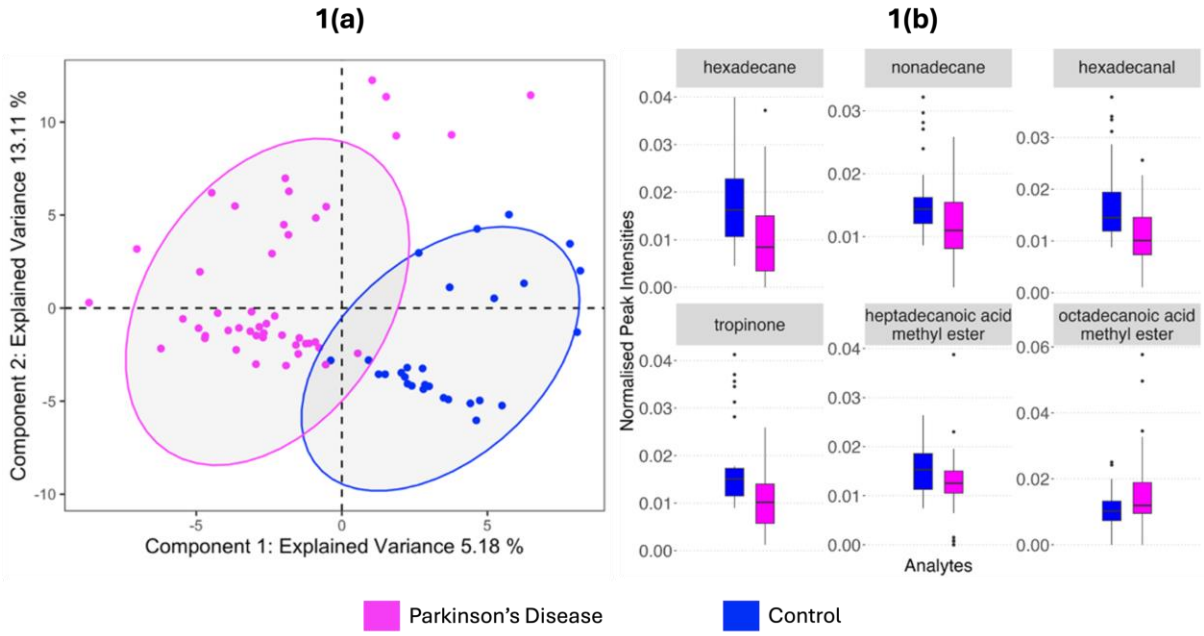

Figure 1: *Modelling VOC profiles from skin swabs of Parkinson's disease and control participants. (a) Optimised sPLS-DA showed two clusters distinctly separating PD and controls, validated by 3-fold cross validation with 100 repeats. (b) Box plots for six of the 85 significant features (alkanes and FAMES) to compare mean expression in PD and controls, selected using VIP scores (>1) and cross validation feature stability scores (>0.8).*

Classification of iRBD from control and PD

We examined Austrian cohort and used all 613 features from TD-GC-MS analysis. The scores plot of sPLS-DA (Figure 2a) shows 3 distinct groups (cross validated with error rate of 0.06). The control participants cluster the most tightly and thus show the least variability between individuals, while more within-group variability in iRBD and PD. Examples of putatively identified features displaying mean iRBD regulation between that of PD and control groups are displayed in Figure 2b.

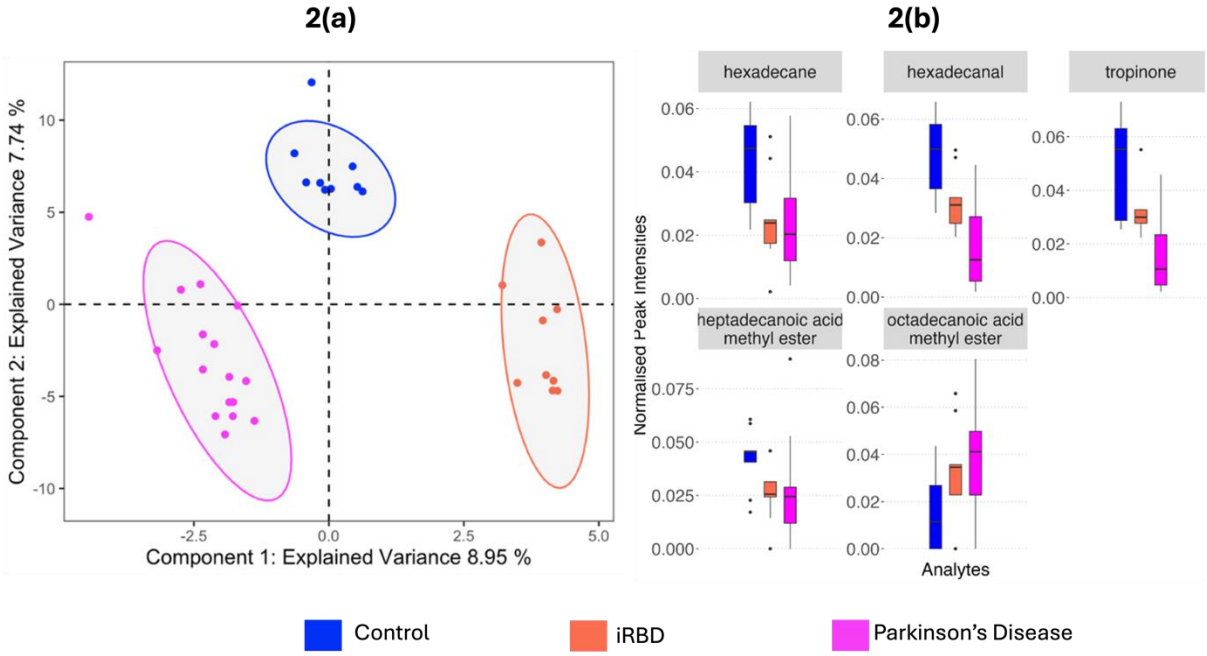

Figure 2: *Modelling VOC profiles collected from skin swabs of PD, control and iRBD participants from the Austrian cohort. (a) Optimised sPLS-DA showed three clusters distinctly separating PD, controls and iRBD participants validated by 3-fold cross validation with 100 repeats. (b) Examples of significant features selected using VIP scores (>1) and cross validation feature stability scores (>0.8) to compare mean expression in PD, controls and iRBD, are shown with box plots.*

Of the 613, 76 features had VIP score > 1 (within component 1 and 2) and cross validation feature stability score > 0.8. Out of these 76 features, 55 showed the mean iRBD concentration to be between that of PD and control, suggesting possible progression features. Nineteen of these 55 possible progression features could be putatively identified (MSI level 2) and these were: 7 alkanes (different regulations), 8 FAMEs (different regulations), two annotations of tropinone (higher in control), an aldehyde (higher in control) and purine (higher in PD). Five of those annotated features are shown in Figure 2b.

Odour signature of iRBD can be distinguished from PD and odour analysis also indicates progression

All iRBD samples and three PD and three control samples were subjected to odour analysis as described previously.[13] The supersmeller, who was not told how many of each pheonotype was presented, was able to distinguish PD from control in all cases and also classify the six of the iRBD samples as possessing a distinct odour; classifying these separately. Three of the iRBD samples were

assigned as PD by the supersmeller. Two out of these three participants classified as PD showed signs of conversion to PD at clinical follow ups post sampling. This observation indicates that the odour of each disease can be distinguished from control, that iRDB has a distinct volatilome, and also that there are volatile markers that may report on the conversion/progression to PD.

VOC signature with Parkinson's disease progression – longitudinal data

When comparing the volatile features found from the same individuals sampled at yearly intervals skin volatilome exhibits good separation determined by cross validated (error rate of 0.15) sPLS-DA (Figure 3a). The intragroup variation increases over time and tighter clustering is seen at the first year of sampling, indicating is higher variation between individuals as PD progresses. Due to the separation of the timepoints on both component 1 and 2, features with VIP > 1 in either component, as well as stability score > 0.8 were investigated. A total of 38 features were selected for further analysis and annotation.

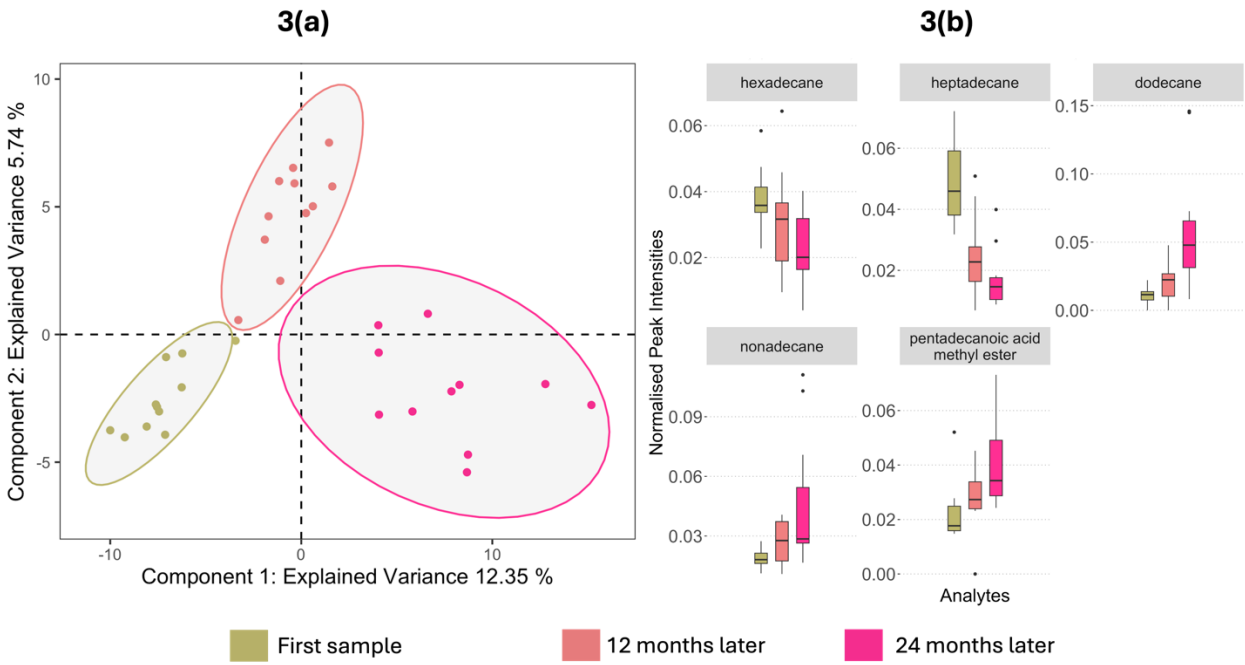

Figure 3: *Modelling VOC profiles collected from skin swabs of PD participants from the UK cohort over three consecutive years. (a) Optimised sPLS-DA showed three clusters distinctly separating PD participants in a trajectory from left to right, validated by 3-fold cross validation with 100 repeats.(b) Significant features consisted of four alkanes and a FAME that showed increasing or decreasing trend as time progresses; selection made using VIP scores (>1) and cross validation feature stability scores (>0.8) to compare mean expression in PD over time.*

Of the 38 features, 22 displayed changes in abundance over the visits, meaning after first 12 months, the abundance was between that found at the time of initial sampling and at 24 months from sampling (Figure 3b). Interestingly none of the features that classify PD (Figure 1 and above) show regulation over the timepoints, implying that the progression features are distinct from diagnostic features. Five of the features that displayed regulation could be assigned a putative identification: four as alkanes (different regulations) and one as a FAME (increasing over the three visits) (Figure 3b).

We sought to determine if these progression features were due to any confounding effects due to age, gender, BMI, medication or comorbidities. We adjusted for known confounders, included them as variables within our models as well as used variables to predict these confounders to ascertain the specificity of these measured metabolites to disease phenotype (Tables S1-S4). All confounders ranked below all metabolites while gender, high cholesterol and heart conditions ranked in the bottom half of the ranking list (Table S2a and S2b). Additionally, none of the models using significant features could predict confounders as an outcome (Table S3) which indicates that these significant features are specific to the phenotypes that were classified, not any potential confounders. We found no strong correlations (>0.5) between significant features and drug intake and measured volatilome was not predictive of any clinical characteristic or drug intake (Table S2c and S4). These analyses overall suggest that the volatilome measured in this study is representative of molecular phenotype of Parkinson's disease and of iRBD and is independent of other clinical history and medication.

Features of Interest

We investigated and interpreted the features of interest in all three comparisons using putative annotations from spectral matches with compound libraries at metabolomics standards initiative (MSI) level 2 confidence.[14]

Lipid Breakdown Products

Changes in fatty acid concentrations in brain tissue,[15] blood[16] and the gut[17] have previously been associated with PD, and we have also reported alterations in lipids with fatty acid chains in sebum.[18] Analysing lipid standards with GC-MS including headspace TD-GC-MS gives rise to several resolvable features identified as alkanes, as opposed to a single identifiable species, as expected. This indicates that ionisation prior to MS analysis may be causing substantial fragmentation.[2] We hypothesise that the alkanes and FAMEs found with high VIP scores and stability scores are derived from larger lipids in sebum. We have demonstrated lipid standards break down in the process of TD-GC-MS analysis, and this combined with the variation in branching and double bond position of the lipid tail groups and endogenous compounds can result in many isomers identified as different features.[2,19] Fatty acids are a well-documented product of the breakdown of larger lipid molecules, and acylglycerols.[20–22] Similarly, aldehydes in headspace may arise due to oxidation of lipids, either endogenously or during the analysis.[23] The inclusion of these as significant in the longitudinal study indicates the lipids alter with disease progression.

Other Putatively Identified Features

Tropinone was identified as significant in both the PD *vs.* control and the iRBD models, the latter of which had two features assigned. It was observed to decrease in PD and show iRBD as the intermediate concentration. It is an alkaloid derived from putrescine.[24] Studies in animal models have shown an important role of putrescine in skin physiology and neuroprotection.[25] Oleamide, an endogenous fatty acid was observed to also be downregulated in PD when compared to controls only, but was not found significantly changing during PD progression or when compared with iRBD. It is a sleep-inducing lipid known to decrease motility in animal models[26,27] though it was not found to be a

feature of interest when classifying iRBD. Purine was found to be upregulated in control *vs*. PD, with intermediate expression in iRBD (supplementary info, Fig S1). One of the end products of purine metabolism is urate - an antioxidant that has been described as a risk marker for PD, and lower levels are also associated with higher severity of disease.[28–30] Alterations in the purine pathway have been researched in an investigation for PD biomarkers, as well as in comparison with other neurological dieases.[31,32] The overall volatilome of iRBD having many components seen as intermediates lends further support to sebum as a reservoir for diagnostic biomarkers of PD. As iRBD can evolve into different disorders (PD, Dementia with Lewy Bodies, Multiple System Atrophy), future work will focus on which of the iRBD-associated features observed in this study are most specific for conversion to either of these conditions.

Conclusions

This study demonstrates the ability of TD-GC-MS to discriminate the volatilome of PD from control using sebum collected on gauze swabs followed by supervised multivariate analysis. When investigating significant features that contribute to classification, we find many metabolite features that can be attributed to lipid breakdown products, validating our previous findings.[2] Such putatively identified markers are not useful for detailed pathway analyses, but furthers our hypothesis that the abundance of lipids captured from sebum changes in the course of the disease.[2] We have previously demonstrated that liquid chromatography-mass spectrometry (LC-MS) can be used to further investigate these larger molecules[18] and here show the power of direct analysis of the volatilome from accessible samples.

Using the same technique and analysing sebum from individuals with iRBD, we observe that a differential volatilome in these participants is present, providing potential for measurement of prodromal molecular markers. Significant features detected in samples from iRBD participants showed presence with a mean concentration between PD and control samples, indicative of disease progression. Finally, we analysed the volatile metabolites in the headspace of samples taken at yearly intervals to investigate changes over disease course and can demonstrate clear classification of samples based on stage of disease. Significant features putatively annotated as hydrocarbons and FAMEs support the hypothesis of differential regulation of lipids as the disease progresses and offer a route to discover markers that could be used to inform the development and efficacy of new treatments.

References

1. Trivedi, D. K. *et al.* Discovery of Volatile Biomarkers of Parkinson's Disease from Sebum. *ACS Cent. Sci.* **5**, 599–606 (2019).
2. Sinclair, E. *et al.* Validating differential volatilome profiles in Parkinson's disease. *ACS Cent. Sci.* **7**, 300–306 (2021).
3. Ray Dorsey, E. *et al.* Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **17**, 939–953 (2018).

4. Poewe, W. *et al.* Parkinson disease. **3**, 1–21 (2017).
5. Tolosa, S. *et al.* Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol.* **20**, 385–397 (2021).
6. Postuma, R. B. & Berg, D. Prodromal Parkinson's Disease: The Decade Past, the Decade to Come. *Mov. Disord.* **34**, 665–675 (2019).
7. Mahlknecht, P., Marini, K., Werkmann, M., Poewe, W. & Seppi, K. Prodromal Parkinson's disease: hype or hope for disease-modification trials? *Transl. Neurodegener.* **11**, 1–13 (2022).
8. Högl, B., Stefani, A. & Videnovic, A. Idiopathic REM sleep behaviour disorder and neurodegeneration - an update. *Nat. Rev. Neurol.* **14**, 40–55 (2018).
9. Boeve, B. F. REM sleep behavior disorder. *Ann. N. Y. Acad. Sci.* **1184**, 15–54 (2010).
10. Miller, I. N. & Cronin-Golomb, A. Gender differences in Parkinson's disease: Clinical characteristics and cognition. *Mov. Disord.* **25**, 2695–2703 (2010).
11. Chambers, M. C. *et al.* A cross-platform toolkit for mass spectrometry and proteomics. *Nat. Biotechnol.* **30**, 918–920 (2012).
12. Domingo-Almenara, X. *et al.* ERah: A Computational Tool Integrating Spectral Deconvolution and Alignment with Quantification and Identification of Metabolites in GC/MS-Based Metabolomics. *Anal. Chem.* **88**, 9821–9829 (2016).
13. Morgan, J. Joy of super smeller: sebum clues for PD diagnostics. *Lancet Neurol.* **15**, 138–139 (2016).
14. Sumner, L. W. *et al.* Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **3**, 211–221 (2007).
15. Fabelo, N. *et al.* Severe alterations in lipid composition of frontal cortex lipid rafts from Parkinson's disease and incidental Parkinson's disease. *Mol. Med. Camb. Mass* **17**, 1107–1118 (2011).
16. Shin, C., Lim, Y., Lim, H. & Ahn, T. B. Plasma Short-Chain Fatty Acids in Patients With Parkinson's Disease. *Mov. Disord.* **35**, 1021–1027 (2020).
17. Unger, M. M. *et al.* Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat. Disord.* **32**, 66–72 (2016).
18. Sinclair, E. *et al.* Metabolomics of sebum reveals lipid dysregulation in Parkinson's disease. *Nat. Commun. 2021 121* **12**, 1–9 (2021).

19. Michael-Jubeli, R., Bleton, J. & Baillet-Guffroy, A. High-temperature gas chromatography-mass spectrometry for skin surface lipids profiling. *J. Lipid Res.* **52**, 143–51 (2011).
20. Smith, R. N., Braue, A., Varigos, G. A. & Mann, N. J. The effect of a low glycemic load diet on acne vulgaris and the fatty acid composition of skin surface triglycerides. *J. Dermatol. Sci.* **50**, 41–52 (2008).
21. Marzouki, Z. M. H., Taha, A. M. & Gomaa, K. S. Fatty acid profiles of sebaceous triglycerides by capillary gas chromatography with mass-selective detection. *J. Chromatogr. B. Biomed. Sci. App.* **425**, 11–24 (1988).
22. Shetewi, T. *et al.* Investigation of the relationship between skin-emitted volatile fatty acids and skin surface acidity in healthy participants—a pilot study. *J. Breath Res.* **15**, 037101 (2021).
23. Shahidi, F. Headspace volatile aldehydes as indicators of lipid oxidation in foods. *Adv. Exp. Med. Biol.* **488**, 113–123 (2001).
24. Bedewitz, M. A., Jones, A. D., D'Auria, J. C. & Barry, C. S. Tropinone synthesis via an atypical polyketide synthase and P450-mediated cyclization. *Nat. Commun.* **9**, (2018).
25. Jänne, J. *et al.* Animal disease models generated by genetic engineering of polyamine metabolism. *J. Cell. Mol. Med.* **9**, 865–882 (2005).
26. Boger, D. L. *et al.* Exceptionally potent inhibitors of fatty acid amide hydrolase: The enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 5044–5049 (2000).
27. Roy, A., Kundu, M., Chakrabarti, S., Patel, D. R. & Pahan, K. Oleamide, a Sleep-Inducing Supplement, Upregulates Doublecortin in Hippocampal Progenitor Cells via PPAR α. *J. Alzheimers Dis.* **84**, 1747–1762 (2021).
28. Chen, X., Wu, G. & Schwarzschild, M. A. Urate in Parkinson's Disease: More Than a Biomarker? *Curr. Neurol. Neurosci. Rep.* **12**, 367–375 (2012).
29. Weisskopf, M. G., O'Reilly, E., Chen, H., Schwarzschild, M. A. & Ascherio, A. Plasma Urate and Risk of Parkinson's Disease. *Am. J. Epidemiol.* **166**, 561–567 (2007).
30. Cipriani, S., Chen, X. & Schwarzschild, M. A. Urate: A novel biomarker of Parkinsons disease risk, diagnosis and prognosis. *Biomark. Med.* **4**, 701–712 (2010).

31. Constantinescu, R., Andreasson, U., Holmberg, B. & Zetterberg, H. Clinical Commentary Serum and cerebrospinal fluid urate levels in synucleinopathies versus tauopathies. *Acta Neurol Scand* **127**, 8–12 (2013).
32. Koros, C. *et al.* Serum uric acid level as a putative biomarker in Parkinson's disease patients carrying GBA1 mutations: 2-Year data from the PPMI study. *Parkinsonism Relat. Disord.* **84**, 1–4 (2021).